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Short Communication

Effect of herbicides on phase transitions of dipalmitoyl phosphatidylcholine vesicles

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The gel to liquid-crystalline phase transition of dipalmitoyl phosphatidylcholine (DPPC) vesicles was monitored with a light-scattering technique in the presence and absence of herbicides. The phase transition temperature for pure DPPC was determined as 41.6°C. 2-Methoxy-3,6-dichlorobenzoic acid (dicamba), 2-methoxy-3,6-dichlorophenylacetic acid (methoxy fenac), 2,4,5-T, (2,4,5-trichlorophenoxy) acetic acid (2,4,5-T), (2,4-dichlorophenoxy) acetic acid (2,4-D), 2-(2,4,5-trichlorophenoxy) propionic acid (silvex), and 4(2,4-dichlorophenoxy) butyric acid (2,4-DB) caused a depression in the phase transition temperature ($-\Delta T$), whose values increased in the following order: 0.6, 3.0, 3.8, 4.6, 7.6 and 9.6°C, respectively. A linear relation was observed between the $-\Delta T$ values and log K (partition coefficient) values for the tested herbicides. This suggested that the perturbation induced in the membrane bilayers by the herbicides was related to the lipid solubilities of the herbicides.

Keywords: phenoxy herbicides; phase properties; phospholipid vesicles; partition coefficients; membrane lipids.

Introduction

Membrane lipids play an important role in maintaining the normal function and structure of biomembranes in plant and animal cells [1]. However, studies with selected herbicides indicate that these substances interfere with plant membrane functions [2]. Leakage of solutes and ions from plant cell membranes is a common occurrence after treatment with various herbicides [3]. An interaction between herbicides and membrane lipids has long been suspected of initiating the biochemical breakdown of plant membranes.

Studies with model membranes have provided additional evidence for a herbicide-lipid interaction. Increases in the permeability of phosphatidylcholine liposomes to hydrogen ions have been observed with the herbicides dinoseb, propanil, ioxynil and several carbanilates [4]. Several phenoxy herbicides have been shown to have a higher affinity for lecithin vesicles in the protonated or unionized form at pH 3.0 than in the ionized form at pH 5.5 and 9.8 [5].

Studies on membrane fluidity have provided much information on the effects of various substances on the phase properties of membrane lipids [6]. Heat energy absorbed by membrane vesicles causes the phospholipid membrane to undergo a gel to liquid-crystalline phase transition that is associated with a molecular rearrangement of the phospholipid's fatty acyl chains. A number of solutes, such as cholesterol [7], vitamin E [8] and surfactants [9] are capable of altering the phase transition temperatures of phospholipid vesicles.

In the present study, changes in the phase transition temperature of dipalmitoyl phosphati-

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dylcholine (DPPC) vesicles caused by herbicides were monitored with a light scattering technique. There are no reports in the literature describing the effect of herbicides on the phase properties of phospholipid membranes.

Experimental section

Materials

Synthetic dipalmitoyl phosphatidylcholine (approx. 99% purity) was obtained from Sigma Chemical Co. (St. Louis, MO). The following herbicides were commercially purchased and used without further purification: 2-methoxy-3,6-dichlorobenzoic acid (dicamba), (Velsicol Chemical Corp., Chicago, IL); (2,4,5-trichlorophenoxy) acetic acid (2,4,5-T), (Aldrich Chemical Co., Milwaukee, WI); (2,4-dichlorophenoxy) acetic acid (2,4-D), (Dow Chemical Co., Midland, MI); 2-methoxy-3,6-dichlorophenylacetic acid (methoxy fenac), (Union Carbide Corp., New York, NY); 2-(2,4,5-trichlorophenoxy) propionic acid (silvex), (Union Carbide Corp.); 4(2,4-dichlorophenoxy) butyric acid (2,4-DB), (Union Carbide Corp.). All of the herbicides were analytical reference grade.

Vesicle preparation

A stock suspension of DPPC was prepared by mixing 50 mg with 200 ml of 0.01 M Tris-H-Cl (pH = 6.5). The suspension was sonicated in a cup-horn of a Sonicator (Heat Systems, Model W-380) for 30 min at 225 W, containing water heated between 60°C and 65°C. After cooling the suspension to room temperature, the optical density of the vesicle suspension was measured on a Beckman DU-8 Spectrophotometer with the wavelength set at 400 nm. Most of the optical density measurements fell in the range of 1.1—1.3. The stock suspension was then stored overnight at 4°C. The samples were prepared by combining 0—2 ml of the aqueous solutions containing the various herbicides (conc. = 2.0 mM for herbicides except 2,4-DB which was 1.0 mM because of its low solubility in water) with 8-ml aliquots of the stock suspension of DPPC. Adjustments of the suspensions to pH 3.0 and 6.5 were made with an Orion pH meter. Before the phase transition experiments were conducted, 10-ml sample volumes were heated in water to 60°C, subsequently sonicated in the cup-horn for 1 min and allowed to cool to room temperature.

Method

Aliquots of the samples were poured into cuvettes that were individually placed into a constant temperature cuvette holder connected to a Laude K-4/RD circulating bath. The samples were heated from 30°C to 45°C at a rate of 1°C per min. The temperatures were measured with a microprobe thermocouple, type (IT-18), connected to a YSI Model 42SC Tele-thermometer. The gel to liquid-crystalline phase transition (T_m) of DPPC vesicles was followed optically by monitoring changes in the scattered light intensity as a function of temperature. The light scattered by the various samples were measured with a McPherson Fluorescence Spectrophotometer in the 90° light-scattering mode with both the excitation and emission wavelengths set at 400 nm.

Results and Discussion

The thermotropic phase transitions of pure DPPC vesicles and DPPC vesicles containing 2,4-D are shown in Fig. 1. The phase transition temperature (T_m), which was taken at the midpoint of the normalized curve, was determined as 41.6°C for pure DPPC. This T_m value is in good agreement with T_m values reported by others using different techniques (e.g., 41.3°C with differential scanning/calorimetry [14] and 41.0°C with an earlier light scattering technique [9]. 2,4-D lowered the phase transition temperature of DPPC vesicles to 37.0°C.

Table I shows the depressions of the phase transition temperature ($-\Delta T$) of DPPC vesicles, which were derived from the equation, $\Delta T = T_m - T_{m,o}$ where $T_{m,o}$ represents T_m without the herbicides. The data show that DPPC vesicles containing 2,4-DB had the highest $-\Delta T$ value of 9.6°C, whereas those vesicles containing 2,4-D had a lower $-\Delta T$ value (i.e., two orders of magnitude smaller than 2,4-DB). A comparison of the molecular structures of the herbicides in Table I reveals that 2,4-DB has two more methylene groups than 2,4-D and the structural difference may account for the observations witnessed here.

In an earlier study with a series of organic com-

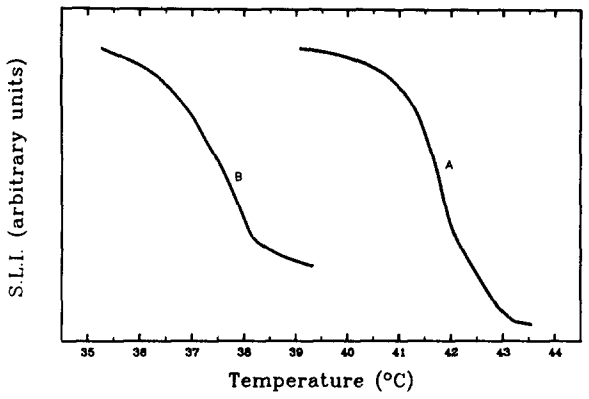
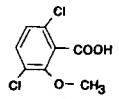
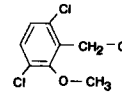
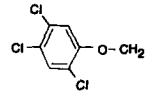
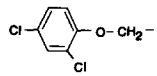
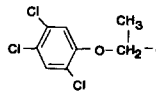
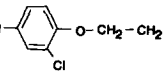


Fig. 1. Tracing of typical scattering light intensity (S.L.I.) scan for pure DPPC vesicles (A) and for DPPC vesicles containing 0.4 mM 2,4-DB (B) as a function of temperature.

pounds differing by one $-CH_2-$ unit, e.g., 1,5-pentanediol and 1,6-hexanediol, Diamond and Wright [10] showed that each successive methylene group increased the oil/water partition coefficients 3.4 times. In Table I, the partition coefficient for 2,4-DB is much greater than the partition coefficient for 2,4-D. Although methods are available for measuring partition coefficients for solutes in membrane vesicles, partition coefficients in the present study were derived from the regression equation $\log K = 5.0—0.67 \log S$, where K = *n*-octanol/water partition coefficient, S = aqueous solubility of the solute in μmol/l and the log is to base 10. This relation between water solubility and partition coefficient was reported by Chiou et al. [11], who showed excellent agreement between calculated and measured *n*-octanol/water partition coefficients for many pesticides. Calculations were made from the regression equation $\log K = 5.0—0.67 \log S$. A plot of $\log K$ values versus $-\Delta T$ values in Fig. 2 shows a linear relation between the calculated partition coefficients of the tested herbicides and the depressions in the phase transition temperature of DPPC vesicles containing herbicides. These results suggest that the perturbation induced in the bilayers is related to the lipid solubilities of the herbicides. A correlation is known to exist between partition coefficients for some non-electrolytes and their permeability coefficients in model membranes [12].

TABLE I

Summary of phase transition data, water solubilities and partition coefficients of herbicides.

Herbicide in DPPC	Structure	T_m (°C)	$-\Delta T$	Solubility in water ppm[b]	Log K[c]
Dicamba[a]		41.0	0.6	4,500 (4,300)	2.1
Methoxy Fenac		38.6	3.0	(2,400)	2.3
2,4,5-T		37.8	3.8	240	3.1
2,4-D		37.0	4.6	890	2.6
Silvex		34.0	7.6	140	3.2
2,4-DB[d]		32.0	9.6	50	3.5

[a]The concentrations of herbicides were 0.4 mM except for 2,4-DB, which was 0.1 mM. 2,4-DB concentration was limited by its low water solubility. The standard deviations for T_m values obtained from duplicate determinations ranged between ± 0.10 and ± 0.48, averaging ± 0.29.

[b]Solubility data were obtained from the literature [13]. Values in parentheses were determined in this laboratory.

[c]Log K (partition coefficient) values were calculated from regression equation $\log K = 5.0 - 0.67 \log S$. K is the *n*-octane/water partition coefficient, S is the aqueous solubility of the solute and log is to base 10.

[d]In respect to mammalian toxicity, oral LD_{50} values for dicamba and 2,4-DB in rats are 1030 and 1960 mg/kg, respectively.

The data in Table II show the effect of pH on the phase transition temperature of DPPC vesicles containing herbicides. The lower pH depressed the phase transition temperature of DPPC more than the higher pH. Based on the dissociation constants (pK_a) for these weak organic acids [13], it is the unionized form of the herbicides that predominantly exist. DPPC presumably is unaffected by the lower pH because phosphatidylcholines are zwitterionic over a wide pH range, (i.e., pH 3—9).

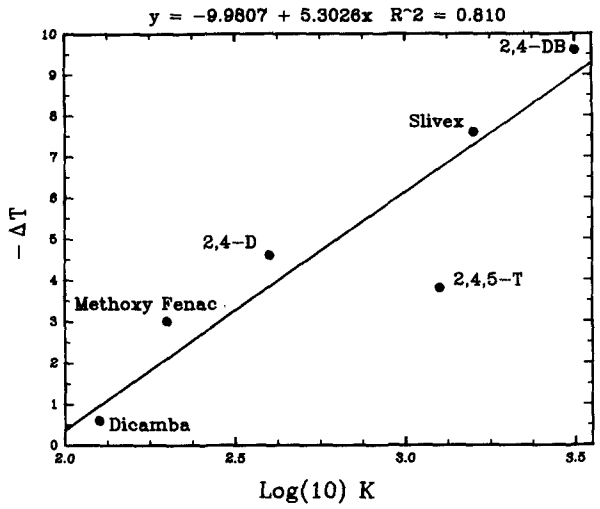


Fig. 2. Plot of $-\Delta T$ values for DPPC vesicles containing herbicides as shown in Table I.

In summary, a simple light-scattering technique has been used to observe the phase transitions of DPPC vesicles. For pure DPPC, the method gave results similar to those obtained with more elaborate techniques, (e.g., differential scanning calorimetry). The phase transition temperature of DPPC was significantly altered by the presence of herbicides in this study. The alteration in the phase transitions is probably due to perturbation induced in the bilayers from a physical interaction between the herbicides and the fatty acyl chains of the membrane phospholipid.

Since biological membranes exists mainly in the liquid-crystalline phase [1], herbicides that enter plant cell membranes may alter the balance between gel phase lipids and liquid-crystalline phase lipids that is necessary for normal membrane function at physiological temperatures. An increase in the proportion of gel phase lipids could probably be disruptive to the structure of cell membrane because bilayers of gel phase lipids have higher permeability rates to ions than bilayers of liquid-crystalline lipids, consistent with the fact that leakage of solutes and ions from plant cell membranes occurs after treatment with various herbicides.

TABLE II

Effect of pH on phase transition temperature (T_m) of DPPC vesicles in the presence of herbicides.

Herbicide in DPPC vesicles[a]	pK_a[b]	pH	T_m (°C)
0	—	6.5[c]	41.6[c]
		3.0	41.6
2,4-D	2.80	6.9	41.4
		3.0	37.0
2,4-DB	4.58	6.9	41.5
		3.0	32.5
2,4,5-T	3.46	6.4	41.2
		3.0	37.8
Silvex	3.1	6.4	41.6
		3.1	34.0
Dicamba	1.94	6.8	41.6
		3.0	41.0
Methoxy fenac	—	6.8	41.2
		3.0	38.6

[a]The concentrations of the herbicides were 0.4 mM except for 2,4-DB, which was 0.1 mM.
[b]pK_a values were obtained from the literature [13].
[c]Values are the means of duplicate determinations.

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Mention of companies or commercial products does not imply recommendation or endorsement by the United States Department of Agriculture over others not mentioned.

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